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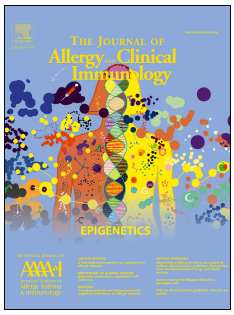

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Dendritic cell recognition by ILC3 via DNAM-1 triggers pro-inflammatory reciprocal cell activations

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Capsule summary:

Human DCs and ILC3 can engage in a reciprocal cross-talk resulting in a bidirectional cell activation relevant either in the regulation of mucosal immunity or in promoting pathological processes such as inflammatory hyperproliferative skin diseases.

Key words: ILC3, DCs, DNAM-1, IL-1β, Psoriasis, Human

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To the Editor

Innate lymphoid cells (ILCs) represent a heterogeneous family of lymphocytes that includes, beside classic cytotoxic natural killer (NK) cells, the more recently described non-cytotoxic ILC populations.

We have previously shown that human NK cells are able to recognize dendritic cells (DCs) by NKp30 receptor, a member of Natural Cytotoxicity Receptor (NCR) family, and that this recognition results in the activation of NK cells[1] and in a process of DC "editing" able to select DCs with the most efficient antigen presenting capability[2-3]. Our current results unveil a similar, but even more effective, bridge between innate and adaptive immunity where group 3 ILC, potent effectors of the very early immune response, can receive an activating signal by DCs via cellular ligands able to engage the activating receptor DNAM-1, and in turn act as early and strong activator of DCs.

Group 3 ILC, also known as ILC3, are characterized by the expression of the transcription factor RORγt. In addition to RORγt, they express homogenously the IL-7 receptor (IL-7R or CD127), the stem cell factor (SCF) receptor c-kit (or CD117) and can be further dissected according to the expression of NKp44 (Fig. E1A).

DNAX Accessory Molecule-1 (DNAM-1) is known to be expressed on NK cells, monocytes and subsets of T cells and has recently described also on the surface of mouse ILC3[4]. We have here shown that human ILC3 express DNAM-1 at levels comparable to NK cells (Fig. 1A) and provided first evidence that, upon DC/ILC3 interaction, the engagement of DNAM-1 receptor on ILC3 is required for inducing granulocyte-macrophage colony-stimulating factor (GM-CSF) production by ILC3, while neither NKp44 nor NKp30 are involved (Fig. 1B). In turn, GM-CSF released by ILC3 stimulates the release of IL-1β by the interacting DCs (Fig. 1C,D) and, remarkably, this latter cytokine can be responsible for DC maturation in an autocrine and, most likely, paracrine fashion on surrounding DCs, as assessed by the upregulation of co-stimulatory/activation molecules on DC surface (Fig. 1E). Of note, the activation of DCs induced by ILC3 was, at least in this experimental setting, significantly more efficient than the one induced by autologous NK cells (Fig. 1E). On the other hand, IL1-β produced by DCs during their cross-talk with ILC3, plays a relevant role also for ILC3 proliferation and production of IL-22 (Fig. 1F), thus resulting crucial for inducing a reciprocal cell activation during the cross-talk between DCs and ILC3. It is noteworthy that during co-culture with autologous DCs, ILC3 proliferate more vigorously than NK cells, which are known to proliferate upon interaction with DCs[1] (Fig. 1G). The robust proliferation of ILC3 occurring upon interaction with DCs, provides a possible explanation for the accumulation of these innate lymphocytes in secondary lymphoid organs, in mucosal sites and, more in general, at epithelial

barriers, where DCs are present as sentinel cells and abundantly recruited during inflammation. Accordingly, the frequency of NKp44^{pos} ILC3 in human inflamed tonsil apparently correlated with the degree of inflammation and, in vitro, appeared the prominent ILC subset activated by DCs (Fig.E1B-E). Remarkably, we showed that IL-1β producing DCs and ILC3 harbor in the same area of human inflamed tonsils (Fig. 1H), thus suggesting that an early release of IL-1β by DCs encountering ILC3 at these sites would play a central role in the initiation, but also in the maintenance, of inflammatory processes.

Hence, in the context of the early phase of the immune response, the role played by ILC3 in activating the subsequent adaptive response might be more relevant than previously appreciated, particularly in settings where activation of DCs by pathogen/danger signals might be inefficient, including cancers.

Of note, when compared to BDCA3^{+} DCs, BDCA1^{+} DCs induced a stronger ILC3 proliferation and release of IL-22, GM-CSF and IL-8 whereas IL-2, TNFα and IL-17 were not produced by ILC3 upon stimulation by both DC subset (Fig.E2A). The evidence that BDCA1^{+} DCs derived from tonsil (as well as from peripheral blood, Fig.E4A) appear particularly prone to activate human ILC3 is in agreement with their specific higher capability in the production of IL-1β when compared to other human DC subsets[5]. DC/ILC3 interaction also results in the expression of NKp44, accompanied at the same time by the upregulation of NKp46 and again BDCA1^{+} DCs are more potent than BDCA3^{+} DCs in inducing this effect (Fig. E3A-C). We also observed that activation of ILC3 in response to DCs is more prominent in ILC3 expressing NKp44, both in terms of proliferation and cytokine secretion (Fig. 2B), with the exception of GM-CSF, which is released in similar amount by NKp44^{neg} and NKp44^{pos}ILC3 exposed to DCs (Fig. 2B). The evidence that steady state NKp44^{neg}ILC3 can produce, upon interaction with DCs, relevant amount of GM-CSF, is in agreement with a model in which the release of GM-CSF by ILC3 acts as triggering factor for completing the reciprocal activation between DCs and ILC3.

The release of other ILC3-associated cytokines, such as IL-22, is known to be sustained by both IL-1β and IL-23. We observed that, along with the release of IL-1β, the encounter with ILC3 induced the upregulation of IL-23 expression in BDCA1^{+} DCs (Fig. 2C), thus further supporting the strong capability of the latter to boost IL-22 production by the interacting ILC3. In tissues, IL-22 levels are regulated by epithelial derived cytokines such as IL-25 and TSLP, which are able to suppress the production of IL-22 by ILC3[6]. Nevertheless, in case of chronic epithelial damage dampening the production of IL-25 and TLSP, the reciprocal activation between DCs and ILC3 should lead to an exacerbating loop of the initial inflammatory process, potentially playing a critical role in the

maintenance and progression of the starting lesions. At the same time, DC activation would reduce their production of IL-22 binding protein, another factor able to control IL-22 activity[7].

This hypothesis might particularly hold true in inflammatory skin diseases characterized by hyperproliferative epidermis, where ILC3 expressing CCR6 and the skin homing receptor cutaneous lymphocyte antigen (CLA)[8] would migrate to damaged epidermis and engage with BDCA1$^+$ DCs, which are abundantly represented in inflamed skin[9]. At these sites, ILC3/DC cross-talk might initiate a self-maintaining loop of reciprocal activation that results in the expansion of ILC3 as well as in the long-term release of IL-22 responsible for the continuous proliferation of epidermal cells, an event frequently occurring in several chronic inflammatory skin diseases. Our current observation that, upon interaction with ILC3, beside IL-1β, DCs can also increase IL-23 expression, which is recognized as a crucial player in psoriasis pathogenesis, is in agreement with this hypothesis.

On the same line, ILC3 expressing Cutaneous Lymphocyte Antigen (CLA), an homing receptor that facilitates the targeting of lymphocytes to inflamed skin, were abundantly represented in both peripheral blood of psoriatic patients and in inflamed tonsils (Fig. 2D). Remarkably, CLA$^+$ ILC3 can produce high level of GM-CSF and accounted for most of IL-22 producing ILC3 upon interactions with DCs (Fig.2E). In this context, it is worth mentioning that IL-22 production is mainly restricted to ILC3 expressing NKp44, a subset not detectable in the peripheral blood of healthy individuals[8], but considerably accumulated and activated upon interaction with DCs. Moreover, in support of the model here proposed, the inflamed papillary dermis of psoriatic skin reveals a massive presence of both IL-1β producing DCs and ILC3 (Fig.2F).

Our current results represent an advance in understanding ILC3 activating signals as well as their interactions with other players of the innate immunity and might be informative for designing modern immunotherapeutic strategies targeting either DC or ILC activation.

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Legends to Figures

Figure 1

DNAM-1 triggers ILC3/DC reciprocal cell activation via IL-1β

A) DNAM-1 expression analyzed on tonsillar ILC3 and NK cells **B)** GM-CSF staining on ILC3 stimulated with BDCA1$^+$DCs in the presence of indicated blocking antibodies or isotype-matched irrelevant mAb (Ig-CTRL) **C)** Detection of IL-1β mRNA in BDCA1$^+$DCs stimulated or not with ILC3. **D)** IL-1β expression on BDCA1$^+$DCs cultured alone or with ILC3 +/- anti-GM-CSF blocking antibody **E)** CD40 and CD83 expression analyzed on BDCA1$^+$DCs alone, with ILC3 +/- IL-1β blocking mAb, and with NK cells. **F)** Ki67 and IL-22 staining in ILC3 stimulated with BDCA1$^+$DCs +/- anti-IL1β blocking mAb. **G)** Ki67 staining of tonsil ILC3 and NK cells stimulated with BDCA1$^+$DCs. **H)** Histology **(a,b)** and immunohistochemistry **(c-l)** of consecutive tonsil sections showing the presence of CD83$^+$cells (brown) (**c,d**) and the expression of IL-1β (red) (**e**,**f**) at the *epithelial(E)-lymphoid interface (left panels)* and in the *inter-follicular T-cell areas (right panels)* between the B-cell follicles (F). Double staining reveals IL-1β(red)/CD83(brown) co-localization (brick-red) (**g**,**h)** with magnification X1000 in the inset) and, in the same areas, NKp46$^+$cells (blue) endowed with RORγt$^+$ nuclei (brown), highlighted by the white box and magnified in the black insets (**i-l**) (a-h and l:X400; *scale bars 30μm.* i-k:X630; *scale bars 20μm*).

Figure 2

BDCA1$^+$DCs are prone to activate ILC3 and induce strong IL-22 production in skin homing CLA$^+$ILC3

A) CFSE proliferation assay and cytokine production by ILC3 following BDCA1$^+$ or BDCA3$^+$DC stimulation. **B)** Flow cytometry analysis of NKp44^{neg} ILC3 cultured with BDCA1$^+$DCs for the expression of Ki67 proliferation marker and acquisition of NKp44 (upper panels). Cytokine production by NKp44^{pos} and NKp44^{neg} ILC3 upon BDCA1$^+$DC stimulation. **C)** IL-23 mRNA was detected in BDCA1$^+$DCs alone or stimulated with ILC3. **D)** CLA expression on ILC3 subsets derived from inflamed tonsils and peripheral blood (PB) of healthy donors and psoriatic patients. Data are representative of at least 10 patients. **E)** IL-22 and GM-CSF production was assessed in both CLA$^+$ and CLA^{neg} ILC3 upon interaction with BDCA1$^+$DCs. One experiment out of three is shown. **F)** Histology **(a)** and immunohistochemistry **(b-d)** of skin biopsies from patients with

psoriasis showing the inflamed papillary dermis (P) filled with $CD83^+$cells (**b**) and, in the same areas, the expression of IL1-β (inset in b) and a crowding of blue stained $NKp46^+$cells endowed with dark-brown $ROR\gamma t^+$ nuclei (**c**), at higher magnification in **d**, and in the inset. (a-c: X400; *scale bars 30μm.* d: X630; *scale bars 20μm*).

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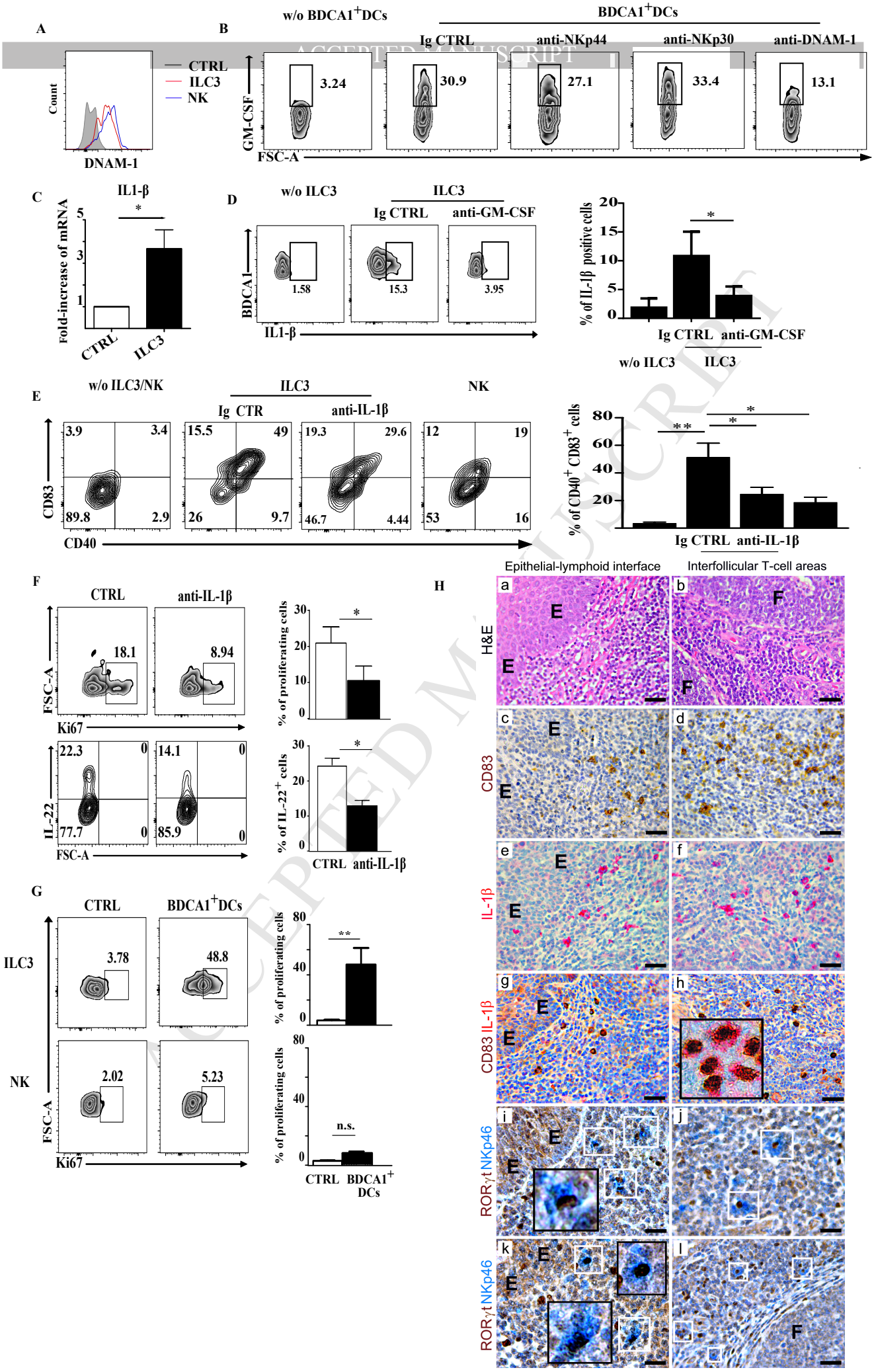

Figure 1

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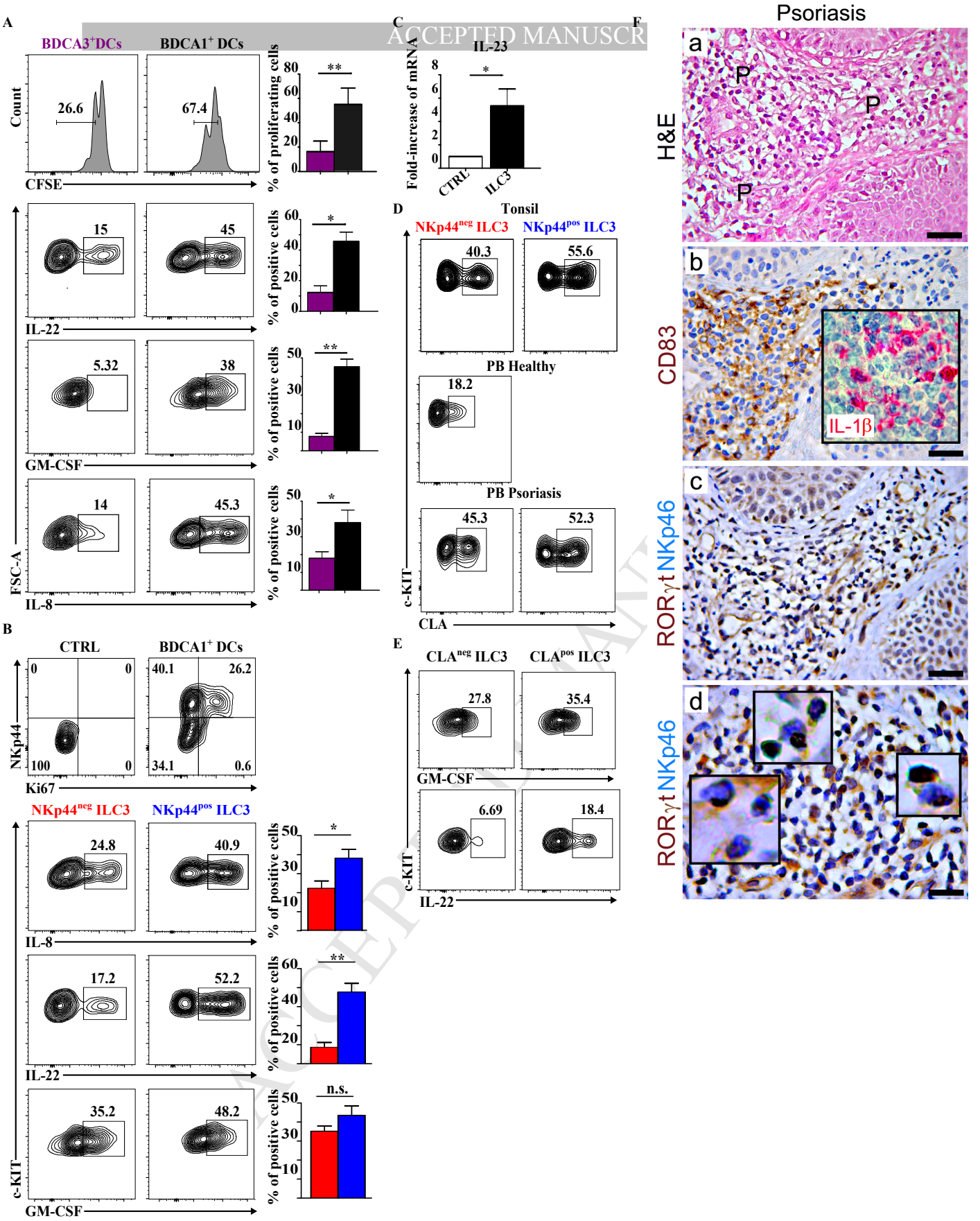

Figure 2

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Methods

Sample Collection

All samples were collected after obtaining informed consent and with approval of tissue-specific protocols by the Ethical Committee of the University Hospital Policlinico *G.Martino*, Messina. Peripheral blood was also obtained from healthy donors and patients affected by active psoriasis. Tonsil tissues were processed as previously described [E1].

Cell Sorting

Total CD127^{hi} ILCs were sorted to high purity (above 98%) by using FACSAria II cell sorter (BD Biosciences) as Lineage (LIN)$^{-}$ (CD3,CD19,CD94,CD14,CD34,BDCA2) CD127^{hi}. Among ILC group 3 ILC were sorted as LIN^{-} CRTH2^{-}cKIT^{+}CD127^{hi}NKp44$^{+/-}$ and ILC1 as LIN^{-} CRTH2^{-}cKIT^{-} CD127^{hi}NKp44^{-}. NK cells were sorted as LIN^{+} NKp46^{+}. Dendritic cells were sorted as LIN^{-} HLA-DR^{+} CD11c^{+} or LIN^{-} HLA-DR^{+} BDCA1^{+} or BDCA3^{+}.

Intracellular cytokine staining

Intracellular staining for the detection of cytokines was performed on freshly isolated tonsillar ILC3 or NK cells stimulated for 18 h with autologous total mDCs or, in selected experiments, with either autologous tonsil or allogeneic blood derived BDCA1^{+} or BDCA3^{+} DCs at ratio 2:1. Monensin (2uM, Sigma-Aldrich) and brefeldin (10ugml^{-1}, Sigma-Aldrich) were added during the last 6 hour of culture to inhibit cell secretion. Detection of intracellular cytokines in blood BDCA1^{+} DCs was performed co-culturing them for 36h with tonsillar NKp44^{pos} ILC3s and monesin and brefeldin were added during the last 12h hours of culture. Cells were then fixed in 1% paraformaldehyde, permeabilized with saponin 0.1% in PBS and stained with the following antibodies: PE-conjugated anti-IL-22, PE-conjugated anti-GM-CSF, PE-conjugated anti-IFNγ, PE-conjugated anti-TNFα, PE-conjugated anti-IL-8 and anti-IL-2, FITC-conjugated anti-IL-1β. Samples were then acquired and about 3x10^{3} events were collected for each subset analysed, using FACS Canto II (BD Biosciences) cytometer and data were analysed by FlowJoVX (Tree Star Inc) software.

Cell Culture Assays

Freshly isolated FACS-sorted human ILCs were plated at $5x10^4$ cellsml^{-1} in RPMI 1640 plus FBS 10% supplemented with Pen/Strep in 96-well round bottom plates. For DC-mediated ILC proliferation, purified LIN$^-$ CD127^{hi} cells were cultured with autologous LIN$^-$ HLADR$^+$ CD11c$^+$ DCs for 6 days. For DC-mediated ILC activation, cells were co-cultured for 48h. For ILC3 and NK cell proliferation experiments, cells were stimulated with autologous tonsillar BDCA1$^+$ DCs, BDCA3$^+$ DCs or, alternatively, blood BDCA1$^+$ DCs at ratio 2:1 for 6 days in the presence of IL-7 (50ngml^{-1}, Miltenyi Biotec). Where indicated, cells were stimulated with recombinant IL-1β (50ngml^{-1}, Miltenyi Biotec). Proliferation was assessed by flow cytometry using CFSE fluorescence or alternatively Ki67 staining. For BDCA1$^+$ DC maturation, purified BDCA1$^+$ DCs from peripheral blood were cultured with freshly isolated ILC3 or NK cells for 24h and then analysed for the expression of CD80, CD83, CD40. The level of expression was measured as the geometric mean of fluorescence (MFI). IL-1β production was assessed on purified blood BDCA1$^+$ DCs cultured for 24h with or without ILC3, by both intracellular staining and Real Time PCR analysis. Alternatively, IL-1β was measured by flow cytometry on BDCA1$^+$ DCs stimulated with 25 ngml^{-1} of recombinant GM-CSF (Sargamostim, Genzyme). In selected experiments neutralizing anti-human IL-1β (clone 2805, R&D), anti-GM-CSF (clone 3209, R&D), anti-NKp44 (IgM, KS38), anti-NKp30 (IgM, F252) and anti-DNAM-1 (IgM, F5) (the last three produced in our laboratories and/or kindly provided by Alessandro Moretta, University of Genoa, Italy) blocking antibodies, or matched isotype controls, were added at the beginning of the cultures.

Quantitative Real Time PCR

mRNA was isolated from cells by using RNeasy MicroKit (QIAGEN). cDNA was synthesized by using Quantitect Reverse Transcription Reagents (QIAGEN) and assayed by qPCR in duplicates by using a Quant Studio DX real-time PCR system (Thermo Fisher Scientific). IL-1β and IL23 TaqMan Gene expression assay (Thermo Fisher Scientific) was employed and mRNA content was normalized to GAPDH expression. Mean relative gene expression was determined by using the DDCT method.

Immunohistochemistry

Formalin-fixed, paraffin-embedded palatine tonsils from pediatric tonsillectomies and skin biopsies from patients with psoriasis were collected from the archives of the Department of Anatomic Pathology, “SS. Annunziata” Hospital of Chieti, Italy

For histology, tonsillar tissue samples were fixed in 4% neutral buffered formalin, embedded in paraffin, sectioned at 4 μm, and stained with hematoxylin and eosin (H&E). For immunohistochemistry, to inhibit endogenous peroxidases, the formalin-fixed, paraffin-embedded sections were treated with 3% H_2O_2 for 5 minutes and, then, washed in H_2O. Antigen was unmasked by heat-induced epitope retrieval in EDTA buffer at pH 8. The slices were then, left at room temperature for twenty minutes. The sections were washed in PBS/Tween-20 and then incubated with the primary antibody (Ab) anti-CD83 Ab (clone 1H4b, Gentaur Srl, Bergamo, Italy), or anti-IL1β Ab (Santa Cruz, Dallas, TX, USA) for 30 minutes. The resulting immune complexes were detected with the Bond Polymer Refine Detection Kit (Leica Biosystems) according to the manufacturer's protocol. Negative controls were performed by replacing the primary antibody with 10% non-immune serum. Double immunohistochemistry was performed, as reported (Di Meo et al., 2014), with anti-CD83 Ab in combination with anti-IL1β Ab, by using the Bond Polymer Refine Red Detection Kit (Leica Biosystems), and anti-NKp46 Ab (clone 195314, R&D, Minneapolis, MN, USA) in combination with anti-RORγt (clone MAB F81, Merck Millipore, Billerica, MA, USA) by using Ferangi Blue Chromogen Kit (Biocare Medical, Concord, CA, USA).

Statistical analysis.

Statistical significance was determined with Student's t-test or linear regression test. Prism GraphPad (GraphPad Software, Inc.) software was used. P-values of <0.05 were considered statistically significant.* = P<0.05, ** = P<0.01.

E-Results

The frequency of ILC3 increases upon culture with DCs and is associated with tonsil inflammation

In human tonsil, ILC3 represent the most prominent subset among $CD127^{hi}$ ILCs accounting for about $6x10^5 \pm 5x10^4$ cells per tonsil, while ILC2 are barely detectable[E2]. (Fig. E1A). Within ILC3 population, ILC3 that express NKp44 is the most abundant subset (Fig. E1A) and, interestingly, we observed that their amount correlated with total peripheral blood leucocyte count, with neutrophilia as well as, in the tonsil, with both the frequency of B cells and the percentage of proliferating B cells within the tonsil (Fig. E1B). Because of this apparent association between inflamed tonsil reactivity and the frequency of $NKp44^{pos}$ ILC3, we hypothesized that accessory cells, such as DCs, might play a role in ILC3 activation and/or proliferation. To evaluate the ability of tonsil-derived

myeloid DCs to instruct ILCs, LIN$^-$ HLA-DR$^+$ CD11c$^+$ cells were sorted from tonsil tissue and co-cultured with autologous CD127^{hi} ILCs. Upon 6 days of co-culture of total CD127^{hi} ILC population with autologous tonsil-derived DCs, the frequency of NKp44^{pos} ILC3 was significantly increased to the detriment of NKp44^{neg} ILC3, whereas no difference was observed for ILC1 (Fig. E1C). In agreement with this data, only NKp44^{pos} ILC3 acquired the expression of Ki67 proliferation marker (Fig. E1D). Confirming their activating ability, tonsil-derived DCs induced an elevated production of IL-22 in autologous ILC3 (Fig. E1E). In contrast to high level of IL-22, tonsillar DCs were, at least in this setting, poor stimulators of IFNγ production by ILC1 and, when we compared tonsillar NK cells and ILC1, the latter produced much lower amount of IFNγ (Fig. E1E).

BDCA1$^+$DCs are more effective than BDCA3$^+$ counterpart in inducing cytokine secretion and conversion from NKp44^{neg} to NKp44^{pos} ILC3

The high frequency of NKp44^{pos} ILC3 observed in human inflamed tonsil (Fig. E1A) might result from the loco-regional proliferation of this ILC subset but also by conversion of NKp44^{neg} ILC3 into NKp44^{pos} cells. Thus, we investigated whether the two distinct subsets of DCs might also display the potential to drive differentiation from NKp44^{neg} ILC3 to the NKp44-expressing counterpart. NKp44^{neg} ILC3 were sorted and cultured for 5 days with DCs or, alternatively, with each of the two DC subsets. Tonsillar myeloid DCs efficiently induced the expression of NKp44 on ILC3, and, again, BDCA1$^+$ DCs were mainly responsible for this induction (Fig. E2A,B). It is noteworthy that the prominent acquisition of NKp44 induced by BDCA1$^+$ DCs was accompanied by a significant up-regulation of NKp46 expression on ILC3 (Fig. E2C).

Functional analyses of ILC populations have shown their major role in the early production of cytokines. ILC3 are able to switch between IL-22 or TNF production, depending on the activating signals involved. Engagement of NKp44 triggers the production of TNFα and IL-2 by ILC3, while cytokine stimulation (IL-23, IL-1β, IL-7) preferentially induces IL-22 and GM-CSF expression[E1, E3]. We evaluated the cytokine production profile of ILC3 upon stimulation with DCs. Remarkably, BDCA1$^+$ DCs were strong inducer of the release of IL-22, IL-8 and GM-SCF by ILC3 while BDCA3$^+$ DCs could only induce the release of a limited amount of IL-22 (Fig.2A). Conversely, IL-2, TNFα and IL-17 were not produced by ILC3 upon interaction with both DC subtypes (Fig. E3A). Then, we investigated the pattern of cytokines induced by DCs on both NKp44^{neg} and NKp44^{pos} ILC3 subsets. As expected, IL-22 production was mainly restricted to the NKp44^{pos} ILC3 cells, IL-8 was significantly higher on NKp44^{pos} compartments, while no differences in GM-CSF production could be observed among the two different subsets upon both BDCA1$^+$ DC (Fig.2B) and BDCA3$^+$ DC stimulation (Fig. E3B).

DCs isolated from peripheral blood are competent for ILC3 activation

In our experimental conditions, tonsillar DCs were able to activate ILC3 in absence of exogenous stimuli able to trigger DC activation. We thus wondered whether a possible explanation could rely on the activated status of DCs, which were isolated from inflamed tonsils. To address this question, BDCA1$^+$ DCs were sorted also from peripheral blood and co-cultured with tonsillar ILC3. Also in this case BDCA1$^+$ DCs could strongly activate ILC3, inducing high amount of cytokines (Fig. E4), thus indicating that tonsil- and blood-derived DCs display similar ILC3 activating properties.

E-References

E1. Carrega P, Loiacono F, Di Carlo E, Scaramuccia A, Mora M, Conte R et al. NCR(+)ILC3 concentrate in human lung cancer and associate with intratumoral lymphoid structures. Nat Commun 2015, 6:8280.

E2. Bernink JH, Peters CP, Munneke M, te Velde A A, Meijer SL, Weijer K et al. Human type 1 innate lymphoid cells accumulate in inflamed mucosal tissues. Nat Immunol 2013,14:221-229.

E3. Glatzer T, Killig M, Meisig J, Ommert I, Luetke-Eversloh M, Babic M et al. RORgammat(+) innate lymphoid cells acquire a proinflammatory program upon engagement of the activating receptor NKp44. Immunity 2013,38:1223-1235.

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Legends to E-Figures

Figure E1

NKp44^{pos} ILC3 accumulated in human inflamed tonsil and proliferate in response to myeloid DCs

Flow cytometry analysis of ILC subset frequency in human tonsils **(A)** and correlation between the frequency of NKp44^{pos}ILC3 and total leucocyte count, percentage of neutrophils, tonsillar B cells and Ki67^{+} B cells **(B)**. Data are from at least 30 different patients. **C)** ILCs were analyzed for the expression of c-KIT and NKp44 by flow cytometry; bar diagrams indicate mean percentage of ILC1, NKp44^{neg} ILC3 and NKp44^{pos} ILC3 cells cultured alone (white bar) or with DCs (black bar) * = P<0.05; n=4. **D**) Intranuclear expression of Ki67 was measured by flow cytometry after gating on the different ILC subsets, as described in figure E1A. Bars represent mean values obtained in 5 experiments. * = P<0.05; ** = P<0.01. **E)** Intracellular cytokine production by FACS-sorted ILC3, ILC1 and NK cells stimulated with DCs. Bars represent mean values of four experiments.

Figure E2

BDCA1^{+} DCs efficiently induce the expression of NKp44 on ILC3

Acquisition of NKp44 and upregulation of NKp46 on FACS-sorted NKp44^{neg} ILC3 cultured with total DCs **(A)** or BDCA1^{+}/BDCA3^{+} subsets **(B,C)**.

Figure E3

Both BDCA1^{+} and BDCA3^{+} DC subsets are poor inducer of TNFα, IL-2 and IL-17 by ILC3 and BDCA3^{+} DCs have a modest effect on IL-22, IL-8 and GM-CSF

A) Intracellular cytokine expression of FACS-sorted ILC3, stimulated as indicated, was measured by flow cytometry. **B**) Intracellular cytokine expression of FACS-sorted NKp44^{pos} and NKp44^{neg} ILC3 upon BDCA3^{+} DC stimulation.

Figure E4

Blood-derived BDCA1^{+} DCs can efficiently activate NKp44^{pos} ILC3

Intracellular cytokine expression of FACS-sorted NKp44^{pos} ILC3 stimulated by BDCA1^{+} DCs isolated from peripheral blood.

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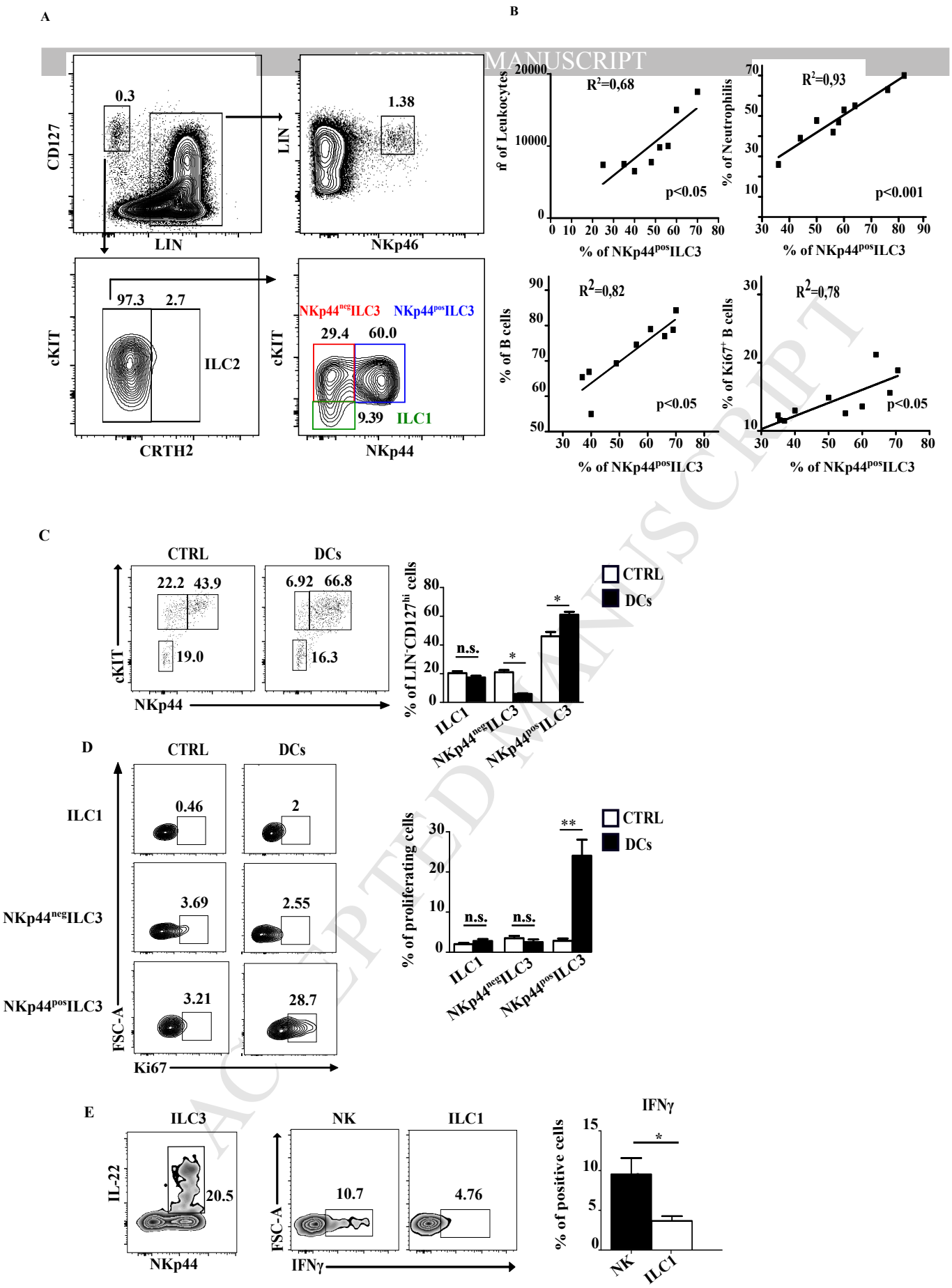

Figure E1

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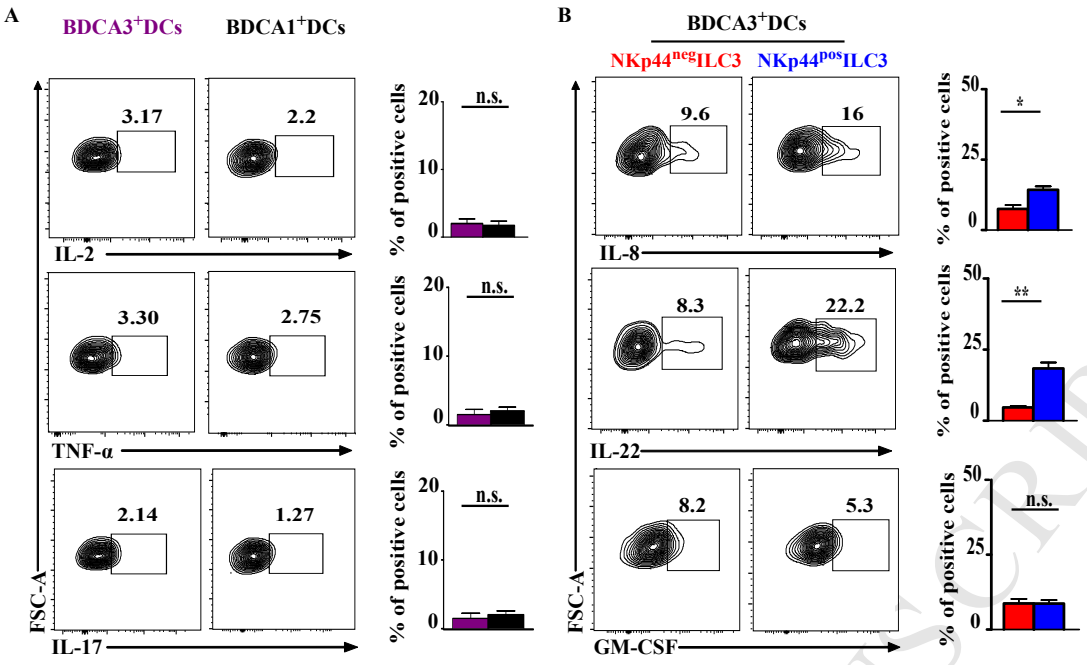

Figure E2

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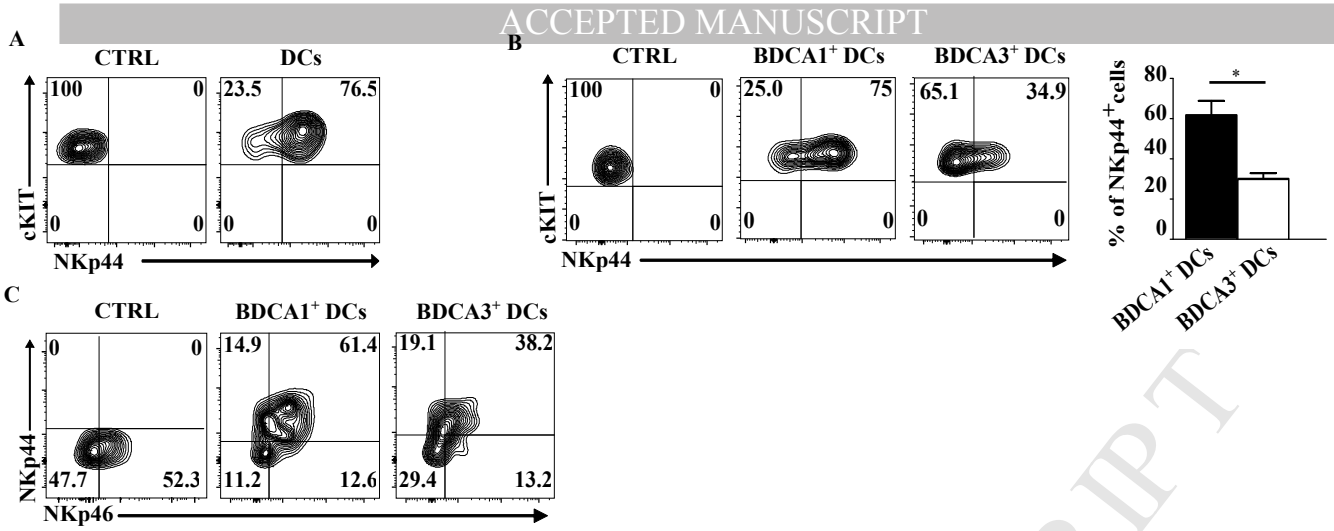

Figure E3

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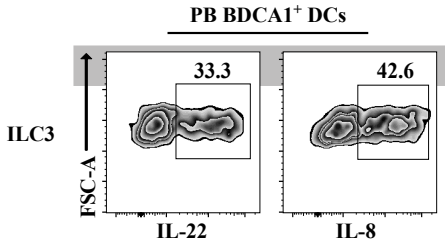

figure E4

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